ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 10, Issue, 01(F), pp. 30640-30643, January, 2019

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

THE EXPRESSION OF COX-2, MUC-1 AND MMP-9 AS PREDICTORS OF SUCCESSFUL PREGNANCY IN PCOS

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DOI: http://dx.doi.org/10.24327/ijrsr.2019.1001.3096

ARTICLE INFO

Article History:
Received 4th October, 2018
Received in revised form 25th November, 2018
Accepted 23rd December, 2018
Published online 28th January, 2019

Key Words:

COX-2, MUC-1, MMP-9, PCOS

ABSTRACT

Background and Aims: PCOS contributes to infertility. The one of infertility cause in PCOS is the poor endometrial receptivity. COX-2, MUC-1 and MMP-9 have an importance role in the embryonic implantation during adhesion, invasion and decidualization. This study intends to know the expression of COX-2, MUC-1 and MMP-9.

Methods: A case control study conducted in FertilitasSekar Clinicand Department of Obstetric Gynecology Moewardi Hospital Central of Java Indonesia. The expression of COX-2, MUC-1 and MMP-9 in endometrial biopsy LH + 5 until LH +10 which meet the inclusion and exclusion criteria was checked by immunohistochemistry. The number of samples were 40 subjects consisting of 20 PCOS patients and 20 fertile women. The data was analyzed using Mann Whitney test.

Results: The mean of COX-2 expression in PCOS (16.25±34.90) in fertile women (42.05±44.15), p=0.065; MUC-1 in PCOS (65.75±44.81), in fertile women (6.80±16.33), p=≤0.001; MMP-9 in PCOS (64.00±34.66), in fertile women (4.15±13.50), p=≤ 0.001.

Conclusion: The expression of COX-2 was not significant. MUC-1 and MMP-9 were higher in PCOS than in fertile women.

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is clinical condition signed by oligoovulation or anovulation, hyperandrogen and description of polycystic ovary.[1] Anovulation is a condition when an ovum does not reach mature follicle causing low rate of successful pregnancy. PCOS becomes the cause of infertility which anovulation is about 40%.The good quality of endometrium signed by receptive endometrium having important role in pregnancy toward women with PCOS.[2] The successful pregnancy in embryo implantation requires harmonious process in apposition, adhesion and invasion of conception result in epithelial endometrium maternal. There are many important factors that localizing interaction between human blastocyst and endometrium before implantation such as, MMP-9, IGF-1, MUC-1, Leptin, VEGF, COX-2, etc. Biomarker such as COX-2, MUC-1, MMP-9 produced by endometrium are cytokines that have an important role for implantation.

This implantation has high contributions as an infertility cause in women with PCOS. Endometrium dysfunction in women with PCOS causes lack of ability in embryo implantation. This means that endometrium cannot produce structure and growth factor needed for embryo implantation.[3] Endometrial receptivity also has an important role in successful pregnancy if there is a change that can reduce the success of assisted reproductive treatment and infertility contribution, like in PCOS.[4] Women with PCOS are sub-fertile that can increase obesity, metabolism, inflammation and endocrine disorder found in Ovulation function, oocyte quality, endometrial receptivity disorders. Endometrium in women with PCOS is supposed as a model of endometrial dysfunction which shows more androgen receptor (AR) and fail to manage estrogen receptor (ER) in implantation window period. The lack of expression in endometrial receptivity and also in regulation and activity of steroid receptor contributes toward low rate of pregnancy in women with PCOS.[5]The successful implantation requires synchronization between the acquisition of implantation competency by blastocyst and receptive condition in the uterine endometrium. These two events are precisely regulated by hormones particularly in estrogen, ovarian and

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progesterone. Molecular and genetic evidence indicates that ovarian hormones together with local molecules including cytokines, growth factor, homeobox transcription factors, lipid mediators, function through autocrine, paracrine and juxtacrine interactions to determine the complex process of implantation.[6] The regulatory roles of progesterone in PCOS with anovulation (oligo –ovulatory) is sub-optimal or absent. This condition results in constant non-opposition of estrogen action in endometrium.[2] Progesterone resistance indicates the decrease of endometrial tissue response toward progesterone. Endometrium with PCOS has impaired progesterone response. In PCOS case, abnormal menstrual cycle and anovulation will present frequently. Endometrium in women with PCOS is thicker than in healthy women.[7]

Subjects and Methods

This research applied analytical observational method with case control study. This research was conducted for 12 months in FertilitasSekarMoewardi Clinic and inObstetric and gynecology department in Moewardi General Hospital Surakarta, Indonesia. Research subjects consisted of population aged 20 – 45years old which are divided into 2 parts, 20 female patients with PCOS and 20 normal women. Sample was taken by fixed diseases sampling. Research variables consisted of dependent variables which were the quality of endometrium in women with PCOS and the quality of endometrium in normal women. Meanwhile, independent variables consisted of the expression of COX-2, MUC-1 and MMP-9 in endometrium. External variables are Profession, age, education, menstrual disorder, the history of PCOS in family, menarche, menstrual cycle, obesity and the history of contraception. The result of data measurement was continuously done by grouping data into categories for analyzing with nominal scale.

The expression of COX-2, MUC-1 and MMP-9 was taken by endometrial biopsy with LH + 5 - LH +10 that required inclusion and exclusion criteria. Next, Immunohistochemical examination was conducted in Pathology Anatomy Department at Sardjito General Hospital Yogyakarta. COX-2 observation was done by using Epitope Specific Rabbit Antibody catalog numbers #RB-9072-R7 (7,0 ml) product of Lab Vision Corporation, USA. Furthermore, MUC-1 applied mouse monoclonal antibody NCL-MUC-1produced by Vision BiosystemNovocastra (NCL-MUC-1). MMP-9 was analyzed using Mouse Monoclonal Antibody MMP-9 produced by Vision BiosystemNovocastra which was specifically used to examine human antigen. The expression of COX-2, MUC-1, MMP-9 was counted based on the observation of cytoplasm epithelial cell consisting 200 cells. Cell positive numbers were counted, then the result was divided by 200 cells multiplied by 100%. The measurement result was expressed as a percentage. Univariate, bivariate and multivariate Analysis were conducted to analyze the data using application of IBM-SPSS statistics 21.

Ethical Clearence

Feasibility of ethics was obtained from the Ethics Commission of Health Research of Dr. Moewardi Hospital/Faculty of Medicine Central Java Indonesia/UniversitasSebelasMaret No. 744/X/HREC/2018 date 29 October 2018

RESULTS

The mean age of PCOS patients was 33.27 ± 5.32 years old divided into two groups. The age with <37 was 16 (80.0%), and the age ≥37 was only 4 (20.0%). In control group, the mean age was 35.6 ± 5.12 years old divided into two groups. The age with <37 was 11 (55.0%), and the age ≥ 37 was 9 (45.0%). The results indicated that based on age group, Most of PCOS patients aged <37 years old with 80% of patients.

Table 1 Characteristic of research subjects

Variable		Group		Total
		PCOS (n=20)	Control (n=20)	
Profession	employed	4 (20.00%)	18 (90.00%)	22 55.00%)
	unemployed	16 (80.00%)	2 (10.00%)	18(45.00%)
Age	<37 years old	16 (80.00%)	11 (55.00%)	27(67.50%)
	≥37 years old	4 (20.00%)	9 (45.00%)	13(32.50%)
Education	primary school/ Junior High school	4 (23.30%)	13 (65.00%)	7 (42.50%)
	Senior high school/ Bachelor	16 (76.70%)	7 (35.00%)	3 (57.50%)

Table 2 Expression of COX-2, MUC-1, MMP-9 in PCOS and Normal

Variable	Mean		p
	PCOS	Normal	
COX-2 (% cell/ field of view)	16.25±34.90	42.05±44.15	0.065
MUC-1(% cell/ field of view)	65.75±44.81	6.80±16.33	<0.001*
MMP-9(% cell/ field of view)	64.00±34.66	4.15±13.50	<0.001*

*Significant p<0.05

The mean of endometrial expression of COX-2in PCOS patients was (16.25±34.90), and in control was (42.05±44.15), with p=0.065; MUC-1 in PCOS patients was (65.75±44.81), and in control group was (6.80±16.33), with p=≤0.001; MMP-9 in PCOS patients was (64.00±34.66), and in control group was (4.15±13.50) with p= ≤ 0.001.

Table 3 Multivariate Linear regression analysis between PCOS effect and External Variablestoward COX-2 expression in endometrium

Independent variable	OR	(CI 95.0%)		p
COX-2 (Model 1)	-25.80	-51.27	-0.32	0.04*
COX-2 (Model 2)	-8.04	-49.29	33.20	0.69
Profession	-10.76	-48.13	26.60	0.56
Age	25.22	-3.87	54.31	0.08
Education	-19.95	-51.44	11.50	0.20
Menstrual Cycle	-3.62	-36.80	29.56	0.82
Family History	-20.68	-57.71	16.35	0.26
Menarche	-34.80	4.01	65.60	0.02*
Menstrual Cycle	-34.74	-73.38	3.90	0.07
Obesity	19.07	-15.94	54.09	0.27
History of contraception	-6.84	-44.32	30.62	0.71

*Significant p<0.05

Multivariate test between external variables and PCOS toward COX-2 expression (table 4) showed that there was a statistically significant correlation. Table 3 Model 1 described that a significant correlation was found statistically in PCOS effect toward COX-2 expression that the expression of COX-2 in PCOS patients decreased -25.80 times with p=0.047, and CI 95% (-51.27 − -0.32). In table 3 model 2, this could be seen that a significant correlation was not shown statistically between PCOS effect and COX-2 expression. By considering all external variables, the expression of COX-2 in PCOS decreased -8.04 times with p=0.69 and CI 95% (-49.29 − 33.20).

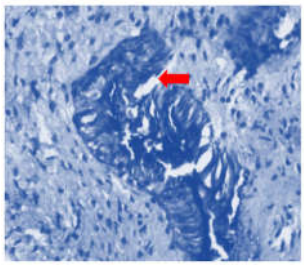

Figure 1 Endometrial expression of COX-2 in normal

Figure 2 Endometrial expression of COX-2 in PCOS

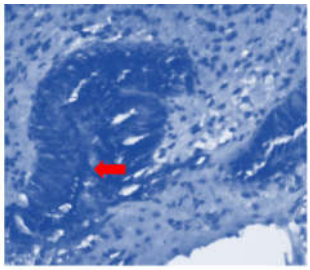

Figure 3 Endometrial expression of MUC-1 in normal

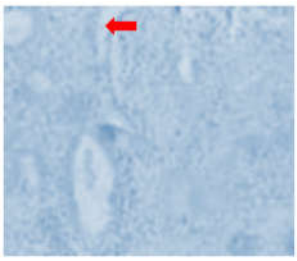

Figure 4 Endometrial expression of MUC-1 in PCOS

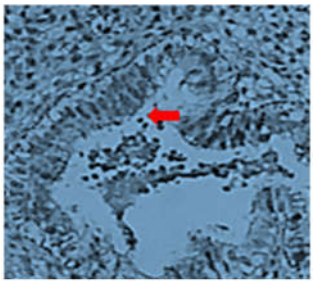

Figure 5 Endometrial expression of MUC-1 in normal.

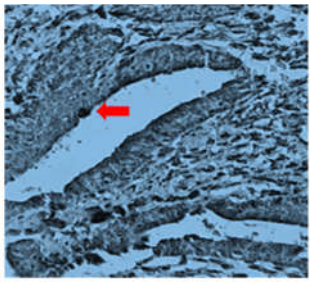

Figure 6 Endometrial expression of MUC-1 in PCOS

Table 4 Multivariate Linear regression analysis between PCOS effect and External Variablestoward MUC-1 expression in endometrium

Independent Variables	OR	(CI 95.0%)		p
MUC1 (Model 1)	58.95	37.35	80.54	0.001*
MUC1 (Model 2)	68.90	32.65	105.15	0.001*
Profession	-36.66	-69.49	-3.82	0.03*
Age	14.12	-11.43	39.69	0.26
Education	7.16	-20.49	34.81	0.60
Menstrual disorder	-4.62	-33.79	24.53	0.74
Family History	24.24	-8.30	56.78	0.13
Menarche	13.72	-13.33	40.78	0.30
Menstrual Cycle	-2.00	-35.96	31.95	0.90
Obesity	37.39	6.62	68.16	0.01*
History of contraception	0.50	-32.42	33.43	0.97

*Significant p<0.05

The result of multivariate test between external variables and PCOS toward MUC-1 expression indicated that there was a statistically significant correlation between the effect of PCOS and MUC-1 expression. In PCOS, the expression of MUC-1 increased 58.95 times with p=≤0.001 and CI 95% (37.35-80.54).Moreover, Table 4 Model 2 Showed that the correlation between PCOS effect and MUC-1 Expression was statistically significant after All external variables were considered. The expression of MUC-1 in PCOS increased 68.90 times with p=0.001 and CI 95% (32.65 – 105.15).

Table 5 Multivariate Linear regression analysis between PCOS effect and External Variablestoward MMP-9 expression in endometrium

Independent Variables	OR	(CI 95.0%)		p
MMP-9 (Model 1)	59.80	43.01	76.69	0.001*
MMP-9 (Model 2)	61.52	27.62	95.42	0.001*
Profession	-9.65	-40.35	21.05	0.52
Age	0.54	-23.3	24.45	0.96
Education	1.74	-24.11	27.61	0.89
Menstrual disorder	5.80	-21.47	33.07	0.66
Family History	-.017	-30.61	30.26	0.99
Menarche	-5.40	-30.70	19.90	0.66
Menstrual Cycle	-5.70	-37.46	26.05	0.71
Obesity	1.70	-27.07	30.47	0.90
History of contraception	-6.56	-37.36	24.23	0.66

*Significant p<0.05

The result of multivariate test between external variables and PCOS toward MMP-9 expression (table 5 model 1) described that there was a statistically significant correlation between the effect of PCOS and MMP-9 expression. In PCOS, the expression of MMP-9 increased 59.80 times with p=≤0.001 and CI 95% (43.01-76.69). After considering all external variables in PCOS, The expression of MMP-9 increased 61.52 times with p=0.001 CI95% (27.62-95.42). This was shown in Table 5 Model 2.

DISCUSSION

Endometrial implantation is controlled by mechanism of complex interaction between embryo and endometrium. Dialogue can occur if there is synchronization between oocyte maturation of endometrium followed by blastocyst orientation into endometrial wall started with apposition, adhesion and invasion into endometrial wall. Embryo implantation is the result of various process which run well including cellular adhesion, invasion and decidualization in endometrium.[4]The mechanism of immune system management is managed through genetic process by ovarian hormones. Basically, Endometrium closely refuses embryo during menstrual cycle. However, endometrium can change, and it possible to have implantation process needed Physiological effort. In the beginning of menstrual cycle, estrogen level increases and this condition also increase endometrial cell proliferation. When the ovulation occurs, progesterone level released by luteinizing follicle tends to have cell differentiation. Therefore, Endometrium is optimal and mature to have embryo implantation.[8] Receptive and functional endometrium is crucial and complex for embryo implantation. During menstrual cycle, endometrium has physiological and morphological change. In that phase, endometrium is prepared to have interaction with embryo in order to get successful implantation. After all biological changes are adequate, embryo is able to do adhesion, endometrial invasion and implantation.[9] Endometrial receptivity is signed physiologically by pinopodes which are present on theephitelial membrane of endometrial surface, as the sign of morphological endometrial receptivity.[5,10] Pinopodes expression is limited in a brief period of maximum 2 days in menstrual cycle during window of implantation. Blastocyst adhesion is shown onto the top of endometrial pinopode.[11]The result showed that COX-2 expression was lower in PCOS than in control group although this was not significant. That result supported the recent research that the decrease of cyclooxygenase expression caused the decrease of endometrial receptivity. This condition can cause recurrent

miscarriages.[9] Estrogen in PCOS causes endometrial dysfunction correlated to the increase of COX-2 level. PCOS patients have endometrial dysfunction as a result of the increase in macrophage activity. A receptive environment in uterine is a requirement for embryo implantation. Endometrium should be in a conducive condition to support adhesion, implantation and placental growth for continuing early pregnancy.[12] There are many factors that localizes interaction between human blastocyst and endometrium before implantation. There are for example, MMP-9, IGF-1, MUC-1, Leptin, VEGF, COX-2.[13] There is a correlation between COX-2 expression and progesterone receptor because embryo will have implantation. As a consequence, COX-2 will disappear followed by the disappearance of progesterone receptor. Concentration of serum progesterone increases and associates the formation of corpus luteum. After more than 8-10 days exposed by progesterone, nuclear progesterone receptor in epithelial uterine will down-regulate. It tends to lose direct effect from progesterone toward type cells of endometrium. If Epithelial synthesis of COX-2 is stimulated by progesterone, Progesterone receptor will lose from epithelial uterine. As a consequence, COX-2 production will decrease and open receptive condition for conception of adhesion.[8] Endometrium environment is managed strongly by both estrogen and progesterone.[14] Estrogen in PCOS patients causes endometrial dysfunction correlating with the increase of MUC-1 expression. PCOS patients have endometrial dysfunction as a result of the increase in macrophage activity.[15] A receptive environment in uterine is a requirement for embryo implantation. Endometrium should be in a conducive condition to support adhesion, implantation and placental growth for continuing early pregnancy. The result of MUC-1 expression in PCOS patients is higher than in control group. This condition causes the difference of endometrial receptivity. This research is in line with another research about MUC-1 expression which is too high in PCOS patients affecting changes of embryo adhesion during implantation into endometrial wall. MUC-1 is one of biomarkers needed to be noted during blastocyst adhesion into endometrial wall. The higher MMP-9 expression in PCOS patients affects changes of embryo adhesion during implantation in endometrial wall. MMP-9 is one of biomarkers needed to be noted during blastocyst adhesion into endometrial wall. At that time, due to long period, endometrial uterine opens toward progesterone stimulation.[10] Concentration of serum progesterone increases and associates the formation of corpus luteum. After more than 8-10 days exposed by progesterone, nuclear progesterone receptor in epithelial uterine will down-regulate. It tends to lose direct effect from progesterone toward type cells of endometrium.[8]The increase of MMP-9 expression in PCOS case causes non-receptive endometrial surface because MMP-9 is anti-adhesive. Then, there is an increase of anti-adhesive. The increase of MMP-9 expression which is more than normal will disturb embryo adhesion into endometrial wall. This condition can cause the need of higher progesterone receptor and disturb embryo adhesion.[8]

CONCLUSION

The expression of COX-2 was not significant. Meanwhile, MUC-1 and MMP-9 were higher in PCOS patients than in fertile woman.

Conflict of Interest Statement

Authors declare that there are no conflicts of interest related to this research, the writer or publication of this article.

Acknowledgement

We extend gratitude to the Director Dr. Moewardi Hospital**,** Dr. Suharto Wijanarko, dr., Sp.U and network hospitals for the permission that we could do the research at the hospital.

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